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Attorney Docket No. 87714/113

In re patent application of

Thyagarajan Srikantha *et al.*

Serial No.: 09/424,951

Group Art Unit: 1636

Filed: January 20, 2000

Examiner: Katharine Davis

For: Candida Albicans Two-Component Hybrid Kinase Gene, CaNIK1 and Use
Thereof.

DECLARATION UNDER 37 CFR § 1.131

I, Thyagarajan Srikantha, declare that:

1. I am a citizen of the United States of America, residing at 1488 Valley View Drive, Coral Village, Iowa 522401.

2. I am employed at the Department of Biological Sciences, at the University of Iowa, Room 314, Iowa City Iowa, 52242

3. I am a co-inventor named in U.S. application Serial No. 09/424,951. In relation to the application, I have reviewed an Office Action, mailed May 24, 2001 and U.S. Patent No. 5,939,306 issued August 17, 1999 cited in that Office Action.

4. Exhibit A, entitled "PCR Amplification to Determine a CaNIK1 Probe," contains notebook entries, with the dates removed, that show the creation prior to April 16, 1997 of a *C. albicans* polynucleotide CaNIK1 "two component histidine kinase homologue" probe, using primers that have homology to two highly conserved regions of "two component response regulator" proteins in bacteria. These same data appear in Example I of the present application.

Serial No. 09/228,958

5. Exhibit B, entitled "Isolation of the CaNIK1 Gene," contains notebook entries that document (1) the identification of the CaNIK1 gene in *E. coli* strain containing the lambda phage ϕ SA15.1, using the probe obtained from the experiments discussed in Exhibit A. These same data appear in Example 2 of the current application.

6. Exhibit C, entitled "Determination of the full-length sequence of the CaNIK1 Gene," contains notebook entries that confirm the acquisition of the nucleotide sequence of the isolated CaNIK1 gene of exhibit B. These same data are shown in Example 2 and Figure 2 A-C of the present application.

7. All experiments described in Exhibits A-C were conducted under my direction and supervision or that of my coinventor, Dr. David Soll.

8. This project was diligently carried out without delay from prior to April 16, 1997 until its completion.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

July 23rd 2001
Dated

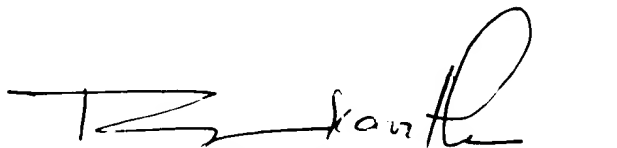

Thyagarajan Srikantha



Exhibit A

PCR Amplification to Determine a CaNIK1 Probe

EXHIBIT
A

Isolation of McMI/SLNI homologues from *C. alb* and *C. glabrata*

McMI oligo's:

Conserved region between *S. cere* and *S. pombe* McMI and NAPI genes containing NADS box. Mc5' and Mc3'

SLNI oligo's:

Designed based on ① Histidine auto phosphorylation domain (SRB1) and ② ATP-binding motif (SRB2) and ③ Aspartyl-phosphate acceptor domain motif (SRB3)

S = SLNI homologues, ~~SLNI~~ Lema, B, BarA were considered. PCR reactions.



94/40/72
15/2/3
40 cycles

only SLNI PCR products seen

Use of Ultima for SLN PCR.

Temperature	1x		
10x buf	10 μ l	40	1 \rightarrow Sc 1+2
25mM MgCl ₂	10 μ l	40	2 \rightarrow CA 1+2
10mM dNTP	8 μ l	32	3 \rightarrow CG 1+2
Sm P ₁ 1	1 μ l	4	4 \rightarrow Sc 1+3
Sm P ₁ 2	2 μ l	8	5 \rightarrow CA 1+2
Ultima	2 μ l	8	6 \rightarrow CG 1+3
H ₂ O	0.5 μ l	2	
	66.5 μ l	270 μ l	

Mini PCR diag. PCR.

	1x	
10x \rightarrow	5	14
MgCl ₂ \rightarrow	4	70
dNTP \rightarrow	0.5	56
P ₁ 1 \rightarrow	1	7
P ₁ 2	1	14
Ult	0.1	14
H ₂ O	1.5	1.5
	38.4	540 μ l

94/45/72°C
1' 1' 2.5'
45 cycles

using GEN system Repeat

94/40/72°C
1.5' 2' 3' 40 cycles

ligation of SLN-PCR product
with T-easy Vector:

	<u>SS_{1,2}</u>	<u>SS_{1,3}</u>	<u>SA_{1,2}</u>	<u>SA_{1,3}</u>	<u>SG_{1,2/1,3}</u>
T-Vector	1 μ l	\longrightarrow			
Insert	2.5 μ l				
10x buf	1.0 μ l				
ligase	1.0 μ l				
H ₂ O	4.5 μ l				

5 μ l transformed into STBL2

X-gal plates
good # of transformants.

Diagnostic PCR with SP6/T7 Prime
125 μ l system.

DNA \rightarrow 2.5 μ l	42x	
10x buf \rightarrow 2.5 μ l	105	
25mM MgCl ₂ \rightarrow 2.0 μ l	84 μ l	
10mM NTP \rightarrow 0.25 μ l	10.5 μ l	
5 μ M SP6 \rightarrow 0.5 μ l	21 μ l	
5 μ M T7 \rightarrow 0.5 μ l	21 μ l	
Taq \rightarrow 0.1 μ l	5.0 μ l	
H ₂ O \rightarrow 17.0 μ l	715 μ l	

\rightarrow 94' / 15' / 3'
72' / 45' / 72' \times
40 cycles

ds sequencing of plasmids

		Template	Primer	
1. PSA1.2/5	→ Forward	2 μ l	—	5
	2 → Reverse	2 μ l	—	
1.2/16	3 → F	2 μ l	—	
	4 → R	2 μ l	—	
2. PCG1.3	5 → REN	3 μ l	0.5 μ l	7.5
	6 → G12	3 μ l	2.0 μ l	6.0
3. PCG2.1	7 → REN1	3 μ l	0.5 μ l	7.5
	8 → G12	3 μ l	2.0 μ l	6.0

10 20 30 40 50 60
TNNNGNAATT GTAATACGAC TCACTATAGG GCGAATTGG CCGACGTCG CATGCCCCG
70 80 90 100 110 120
GCCGCCATGG CGGCCGCGGG AATTCEATTG AATTGAGAAC GCCGGTGAAT GGGATTATTG
130 140 150 160 170 180
GAATGACCCA GTTGTCACTT GATACAGAGT TGACACAGTA CCAACGAGAG ATGTTGTCCA
190 200 210 220 230 240
TTGTGCATAA CTTGGCAAAT TCCTTGTTGA CCATTATAGA CGATATATTG GATATTTCTA
250 260 270 280 290 300
AGATTGAGGC GAATAGAATG ACGGTGGAAC AGATTGATTT TTCATTAAGA GGGACAGTGT
310 320 330 340 350 360
TTGGTGCAAT GAAAACGTTA GCCGTCAAAG CTATTGAAAA AAACCTAGAC TTGACCTATC
370 380 390 400 410 420
AATGTGATTC ATCGTTTCCA GATAATCTTA TTGGAGATAG TTTTAGATTA CGACAAGTTA
430 440 450 460 470 480
TTCTTAACTT GGCTGGTAAT GCTATTAAGT TTAATAAAGA GGGGAAAGTT AGTGTTAGTG
490 500 510 520 530 540
TGAAAAAGTC TGATAAAATG GTGTTAGATA GTAAGTTGTT GTTAGAGGTT TGTGTTAGCG
550 560 570 580 590 600
ACACGGGAAT AGGTATAGAG AAAGACAAAT TGGGATTGAT TTTCGATACC TTCTGTCAAG
610 620 630 640 650 660
CTGATGGTTC TACTACAAGA AAGTTTGGTG GTACTGGCTT ANGCTAATC ACTAGTGAAT
670 680 690 700 710 720
TCGCGGCCGC CTGCAGGTCG ACCATATGGG ANAGCCCCAA CGCGTTGGAT GCATAGCTTG
730 740 750 760 770 780
AGTATCTATA GTGTCACTAA ATAGCTTG.....

Handwritten notes and markings:
- *SL1* (with arrow) above sequence 100-170
- *SL2* (with arrow) above sequence 620-640
- *SL3* (with arrow) above sequence 520-590
- *SP1* (with arrow) above sequence 650-660
- *SP2* (with arrow) above sequence 130-140
- *SP3* (with arrow) above sequence 140-150
- *SP4* (with arrow) above sequence 150-160
- *SP5* (with arrow) above sequence 160-170
- *SP6* (with arrow) above sequence 170-180
- *SP7* (with arrow) above sequence 180-190
- *SP8* (with arrow) above sequence 190-200
- *SP9* (with arrow) above sequence 200-210
- *SP10* (with arrow) above sequence 210-220
- *SP11* (with arrow) above sequence 220-230
- *SP12* (with arrow) above sequence 230-240
- *SP13* (with arrow) above sequence 240-250
- *SP14* (with arrow) above sequence 250-260
- *SP15* (with arrow) above sequence 260-270
- *SP16* (with arrow) above sequence 270-280
- *SP17* (with arrow) above sequence 280-290
- *SP18* (with arrow) above sequence 290-300
- *SP19* (with arrow) above sequence 300-310
- *SP20* (with arrow) above sequence 310-320
- *SP21* (with arrow) above sequence 320-330
- *SP22* (with arrow) above sequence 330-340
- *SP23* (with arrow) above sequence 340-350
- *SP24* (with arrow) above sequence 350-360
- *SP25* (with arrow) above sequence 360-370
- *SP26* (with arrow) above sequence 370-380
- *SP27* (with arrow) above sequence 380-390
- *SP28* (with arrow) above sequence 390-400
- *SP29* (with arrow) above sequence 400-410
- *SP30* (with arrow) above sequence 410-420
- *SP31* (with arrow) above sequence 420-430
- *SP32* (with arrow) above sequence 430-440
- *SP33* (with arrow) above sequence 440-450
- *SP34* (with arrow) above sequence 450-460
- *SP35* (with arrow) above sequence 460-470
- *SP36* (with arrow) above sequence 470-480
- *SP37* (with arrow) above sequence 480-490
- *SP38* (with arrow) above sequence 490-500
- *SP39* (with arrow) above sequence 500-510
- *SP40* (with arrow) above sequence 510-520
- *SP41* (with arrow) above sequence 520-530
- *SP42* (with arrow) above sequence 530-540
- *SP43* (with arrow) above sequence 540-550
- *SP44* (with arrow) above sequence 550-560
- *SP45* (with arrow) above sequence 560-570
- *SP46* (with arrow) above sequence 570-580
- *SP47* (with arrow) above sequence 580-590
- *SP48* (with arrow) above sequence 590-600
- *SP49* (with arrow) above sequence 600-610
- *SP50* (with arrow) above sequence 610-620
- *SP51* (with arrow) above sequence 620-630
- *SP52* (with arrow) above sequence 630-640
- *SP53* (with arrow) above sequence 640-650
- *SP54* (with arrow) above sequence 650-660
- *SP55* (with arrow) above sequence 660-670
- *SP56* (with arrow) above sequence 670-680
- *SP57* (with arrow) above sequence 680-690
- *SP58* (with arrow) above sequence 690-700
- *SP59* (with arrow) above sequence 700-710
- *SP60* (with arrow) above sequence 710-720
- *SP61* (with arrow) above sequence 720-730
- *SP62* (with arrow) above sequence 730-740
- *SP63* (with arrow) above sequence 740-750
- *SP64* (with arrow) above sequence 750-760
- *SP65* (with arrow) above sequence 760-770
- *SP66* (with arrow) above sequence 770-780

10	20	30	40	50	60
ATGGATGACG	CCAAACTATT	TAGGTGACAC	TATAGAATAC	TCAAGCTATG	CATCCAACGC
70	80	90	100	110	120
GTTGGGAGCT	CTCCCATATG	GTCGACCTGC	AGGCGGCCGC	GAATTCACCTA	GTGATTAGGC
130	140	150	160	170	180
CTAAGCCAGT	ACCACCAAAC	TTTCTTGTAG	TAGAACCATC	AGCTTGACAG	AAGGTATCGA
190	200	210	220	230	240
AAATCAATCC	CAATTTGTCT	TTCTCTATAC	CTATTCCCGT	GTCGCTAACA	CAAACCTCTA
250	260	270	280	290	300
ACAACAACIT	ACTATCTAAC	ACCATTTTAT	CAGACTTTTT	CACACTAACA	CTAACTTTCC
310	320	330	340	350	360
CCTCTTTAGT	AAACTTAATA	GCATTACCAG	CCAAGTTAAG	AATAACTTGT	CGTAATCTAA
370	380	390	400	410	420
AACTATCTCC	AATAAGATTA	TCTGGAAACG	ATGAATCACA	TTGATAGGTC	AAGTCTAGGT
430	440	450	460	470	480
TTTTTTCAAT	AGCTTTGACG	GCTAACGTTT	TCAATGCACC	AAACACTGTC	CCTCTTAATG
490	500	510	520	530	540
AAAAATCAAT	CTGTTCCACC	GTCATTCTAT	TCGCCTCAAT	CTTAGAAATA	TCCAATATAT
550	560	570	580	590	600
CGTCTATAAT	GGTCAACAAG	GAATTTGCCA	AGTTATGCAC	AATCGACAAC	ATCTCTCGTT
610	620	630	640	650	660
GGTACTGTGT	CAACTCTGTA	TCAAGTGACA	ACTGGGTCAT	TCCAATAATC	CCATTCCACCG
670	680	690	700	710	720
GCGTTCTCAA	TTCAATCGAA	TTCCCGCGGC	CGCATGGCGG	GCGGGAACAT	GCGACNTCCG
730	740	750	760	770	780
GCCAATTCGC	CCTATANTGA	GTCGTA....

Exhibit B

Isolation of the CaNIK1 Gene

EXHIBIT

B

Screening of C. alb genomic library
for ~~the~~ walking in 5' direction of
the gene.

Dilute 8 μ l / 500 μ l SM.

Use 10 μ l ϕ + 200 μ l cells.
(P2)

5 plates

Probed with 1.384 probe

Diagnostic PCR
Performed

25 +ve clones isolated

250 μ l SM (\sim 15 plaques / spot)

Plated 1:2500 dilution

4 μ l \rightarrow 10 μ l of ϕ + 120 μ l P2

Probe \rightarrow 1.384

Clones #2, #8, #10, #22, #18

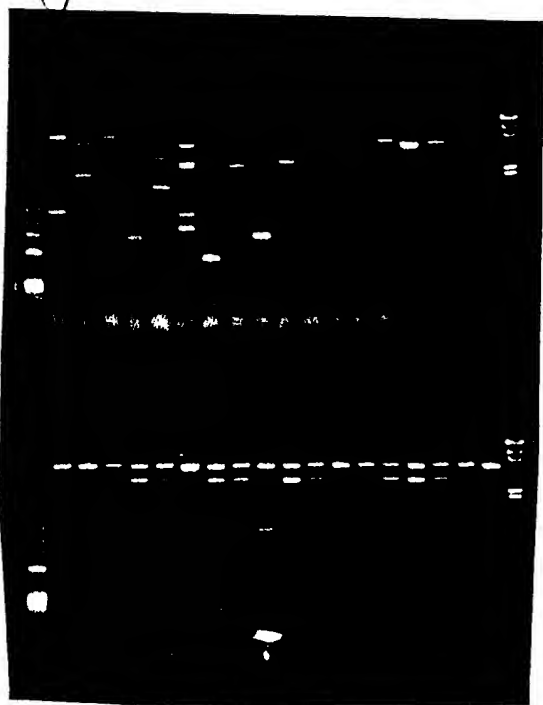
Hybridized ϕ , #15, #1 showed
+ve signals.

Single plaques were re-suspended
in 250 μ l SM.

25 μ l of lysates + 150 μ l P2 were
used to prepare plate lysates

1
2
3
8
10
12
15
18

Diagnostic PCR 1 clone 1 time 18



Top Row $\lambda_{SA1-18} \in ELA + SLB4R$

bot Row $\lambda_{SA1-18} \in ERA + SLB4R$

Long PCR system \in

0.25 μ l Taq + 0.25 μ l long PCR enz

~~Q~~ Total 30 cycles.

Exhibit C

Determination of the Full-Length Sequence of the CaNIK1 Gene

5 9 18 27 36 45 54
ATG GAG TTA ANT TTA AGG GNG AGG GCA GGA GTA TTG TTG GGT GAA ATA GGG GAG
V E L X L R X R A G V L L G E I G E
W S * X * G X G Q E Y C W V K * G S
G V X F K G E G R S I V G * N R G V

63 72 81 90 99 108
TGT GGT GGT TGA GAA GGG AGA GAG TTA TGA AAT TGG GGC ACA GGG TTT TTT TTT
C G G * E G R E L * N W G T G F F F
V V V E K G E S Y E I G A Q G F F F
W W L R R E R V M K L G H R V F F F

117 126 135 144 153 162
TTT TGG TTT TTC CTT TTT TAG TTG GGC ACC AAT ACT AAT TGA TTA TTT TTG CTA
F W F F L F * L G T N T N * L F L L
F G F S F F S W A P I L I D Y F C Y
L V F P F L V G H Q Y * L I I F A I

171 180 189 198 207 216
TTA TGG GCA AAT TGG ATG TGG CAG AAT TTG CAA AAC AGA TCC GGA TAT TGT CAT
L W A N W M W Q N L Q N R S G Y C H
Y G Q I G C G R I C K T D P D I V M
← M G K L D V A E F A K Q I R I L S W

225 234 243 252 261 270
GG CAA GTC AGA AAT TAG TAG GTA TGG CAG TTG GAT GGT GTA GGG TAC TGG CAG
G Q V R N * * V W Q L D G V G Y W Q
← G K S E I S R Y G S W M V * G T G R
← A S Q K L V G M A V G W C R V L A G

279 288 297 306 315 324
GCC CGG TAA AGG AAA CTA AAC ATG TTA GCC AGG GAG TGA GTC CTA CGG GGG GGA
A R * R K L N M L A R E * V L R G G
← P G K G N * T C * P G S E S Y G G E
← P V K E T K H V S Q G V S P T G G S

333 342 351 360 369 378
GCA TAG TAC ACC AGT AGT AGG ACC TCC TTT/GTT GGT CGT GTG TGG TGG CAA TAT
A * Y T S S R T S F V G R V W W Q Y
H S T P V V G P P L L V V C G G N I
I V H Q * * D L L C W S C V V A I Y

387 396 405 414 423 432
ACA AAG CCC CCC CTT ACA TCA ACA AAA AAT TTT TCC TCT CTG TTG TTT ATC TCG
T K P P L T S T K N F S S L L F I S
→ Q S P P L H Q Q K I F P L C C L S R
A → K A P P Y I N K K F L S V V Y L E

441 450 459 468 477 486
GT TTC TCC CTC TCT CTC CAA TGG CAC CCC ACT AAA AAA CCA CGG TTA TCA CCA
S F S L S L Q W H P T K K P R L S P
V S P S L S N G A T P L K N H G Y H Q
→ F L P L S P M A P H * K T T V I T N

SLB 16

SLB 17

495 504 513 522 531 540
ATG CAG CCC TCT GTT TTT GAA ATA CTC AAC GAC CCT GAG CTT TAT AGT CAG CAC
→ M Q P S V F E I L N D P E L Y S Q H
C S P L F L K Y S T T L S F I V S T
A A L C F * N T Q R P * A L * S A L

549 558 567 576 585 594
TGT CAT AGC CTT AGG GAA ACA CTT CTT GAT CAT TTC AAC CAT CAA GCT ACA CTT
→ C H S L R E T L L D H F N H Q A T L
V I A L G K H F L I I S T I K L H L
S * P * G N T S * S F Q P S S Y T Y

603 612 621 630 639 648
ATC GAC ACT TAT GAA CAT GAA CTA GAA AAA TCC AAA AAT GCC AAC AAA GCG TCC
→ I D T Y E H E L E K S K N A N K A S
S T L M N M N * K N P K M P T K R P
R H L * T * T R K I Q K C Q Q S V P

657 666 675 684 693 702
CAA CAA GCA CTT AGT GAA ATA GGT ACA GTT GTT ATA TCT GTT GCC ATG GGA GAC
→ Q Q A L S E I G T V V I S V A M G D
N K H L V K * V Q L L Y L L P W E T
T S T * * N R Y S C Y I C C H G R L

711 720 729 738 747 756
TTG TCG AAA AAA GTT GAG ATT CAC ACA GTA GAA AAT GAC CCT GAG ATT TTA AAA
→ L S K K V E I H T V E N D P E I L K
C R K K L R F T Q * K M T L R F * K
V E K S * D S H S R K * P * D F K S

765 774 783 792 801 810
GTC AAA ATC ACC ATC AAC ACC ATG ATG GAT CAA TTA CAG ACA TTT GCT AAT GAG
→ V K I T I N T M M D Q L Q T F A N E
S K S P S T P * W I N Y R H L L M R
Q N H H Q H H D G S I T D I C * * G

819 828 837 846 855 864
GTT ACA AAA GTC GCC ACC GAA GTC GCA AAT GGT GAA CTA GGT GGA CAA GCG AAA
→ V T K V A T E V A N G E L G G Q A K
L Q K S P P K S Q M V N * V D K R K
Y K S R H R S R K W * T R W T S E K

873 882 891 900 909 918
AAT GAT GGA TCT GTT GGT ATT TGG AGA TCA CTT ACA GAC AAT GTT AAT ATT ATG
→ N D G S V G I W R S L T D N V N I M
M M D L L V F G D H L Q T M L I L W
* W I C W Y L E I T Y R Q C * Y Y G

927 936 945 954 963 972
GCT CTT AAT TTA ACT AAC CAA GTG CGA GAA ATT GCT GAT GTC ACA CGT GCT GTT
→ A L N L T N Q V R E I A D V T R A V
L L I * L T K C E K L L M S H V L L
S * F N * P S A R N C * C H T C C C

```

      981      990      999      1008      1017      1026
GCC AAG GGG GAC TTG TCA CGT AAA ATT AAT GTA CAC GCC CAG GGT GAA ATC CTT
-----
A  K  G  D  L  S  R  K  I  N  V  H  A  Q  G  E  I  L
P  R  G  T  C  H  V  K  L  M  Y  T  P  R  V  K  S  F
Q  G  G  L  V  T  *  N  *  C  T  R  P  G  *  N  P  S

      1035      1044      1053      1062      1071      1080
CAA CTT CAA CGT ACA ATA AAC ACC ATG GTG GAT CAG TTA CGA ACG TTT GCA TTC
-----
Q  L  Q  R  T  I  N  T  M  V  D  Q  L  R  T  F  A  F
N  F  N  V  Q  *  T  P  W  W  I  S  Y  E  R  L  H  S
T  S  T  Y  N  K  H  H  G  G  S  V  T  N  V  C  I  R

      1089      1098      1107      1116      1125      1134
GAA GTA TCT AAA GTT GCT AGA GAT GTT GGT GTG CTT GGT ATA TTA GGA GGA CAA
-----
E  V  S  K  V  A  R  D  V  G  V  L  G  I  L  G  G  Q
K  Y  L  K  L  L  E  M  L  V  C  L  V  Y  *  E  D  K
S  I  *  S  C  *  R  C  W  C  A  W  Y  I  R  R  T  S

      1143      1152      1161      1170      1179      1188
GCG TTG ATT GAA AAT GTT GAA GGT ATT TGG GAA RAR TTG ANT GAT AAT GTC AAT
-----
A  L  I  E  N  V  E  G  I  W  E  X  L  X  D  N  V  N
R  *  L  K  M  L  K  V  F  G  K  X  *  X  I  M  S  M
V  D  *  K  C  *  R  Y  L  G  X  X  X  *  *  C  Q  C

      1197      1206      1215      1224      1233      1242
GCC ATG GCT CTT AAT TTG ANT ACA CAA NTG AGA AAT ATT GCC AAT GTC ACC NCT
-----
A  M  A  L  N  L  X  T  Q  X  R  N  I  A  N  V  T  X
P  W  L  L  I  *  X  H  X  *  E  I  L  P  M  S  P  L
H  G  S  *  F  X  Y  T  X  E  K  Y  C  Q  C  H  X  C

      1251      1260      1269      1278      1287      1296
GCC GTT GCC AAG GGG GAT TTG TCG AAA AAA GTC MCN NCT GAT TGT ANG GGA GAA
-----
A  V  A  K  G  D  L  S  K  K  V  X  X  D  C  X  G  E
P  L  P  R  G  I  C  R  K  K  S  X  L  I  V  X  E  K
R  C  Q  G  G  F  V  E  K  S  X  X  *  L  X  G  R  N

      1305      1314      1323      1332      1341      1350
ATY CTT GAT TTG AAA CTT ACT ATT AAT CAA ATG GTG GAC CGA TTA CAG AAT TTT
-----
I  L  D  L  K  L  T  I  N  Q  M  V  D  R  L  Q  N  F
X  L  I  *  N  L  L  L  I  K  W  W  T  D  Y  R  I  L
X  *  F  E  T  Y  Y  *  S  N  G  G  P  I  T  E  F  C

      1359      1368      1377      1386      1395      1404
GCT CTT GCG GTG ACG ACA TTG TCG AGA GAG GTT GGT ACT TTG GGT ATT TTG GGT
-----
A  L  A  V  T  T  L  S  R  E  V  G  T  L  G  I  L  G
L  L  R  *  R  H  C  R  E  R  L  V  L  W  V  F  W  V
S  C  G  D  D  I  V  E  R  G  W  Y  F  G  Y  F  G  W

      1413      1422      1431      1440      1449      1458
GGA CAA GCT AAC GTA CAG GAT GTT GAA GGT GCT TGG AAA CAG GTT ACA GAA AAT
-----
G  Q  A  N  V  Q  D  V  E  G  A  W  K  Q  V  T  E  N
D  K  L  T  Y  R  M  L  K  V  L  G  N  R  L  Q  K  M
T  S  *  R  T  G  C  *  R  C  L  E  T  G  Y  R  K  C

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1467      1476      1485      1494      1503      1512
GTC AAC CTA ATG GCT ACT AAT TTA ACT AAC CAA GTG AGA TCT ATT GCT ACA GTT
-----
V  N  L  M  A  T  N  L  T  N  Q  V  R  S  I  A  T  V
S  T  *  W  L  L  I  *  L  T  K  *  D  L  L  L  Q  L
Q  P  N  G  Y  *  F  N  *  P  S  E  I  Y  C  Y  S  Y

1521      1530      1539      1548      1557      1566
ACT ACT GCA GTT GCG CAT GGT GAT TTG TCG CAA AAG ATT GAT GGT CAT CCC AAA
-----
T  T  A  V  A  H  G  D  L  S  Q  K  I  D  G  H  P  K
L  L  Q  L  R  M  V  I  C  R  K  R  L  M  V  I  P  K
Y  C  S  C  A  W  *  F  V  A  K  D  *  W  S  S  Q  R

1575      1584      1593      1602      1611      1620
GGA GAG ATT TTA CAA TTG AAA AAT ACA ATC AAC AAG ATG GTG GAC TCT TTG CAG
-----
G  E  I  L  Q  L  K  N  T  I  N  K  M  V  D  S  L  Q
E  R  F  Y  N  *  K  I  Q  S  T  R  W  W  T  L  C  S
R  D  F  T  I  E  K  Y  N  Q  Q  D  G  G  L  F  A  V

1629      1638      1647      1656      1665      1674
TTG TTT GCA TCA GAA GTG TCG AAA GTG GCA CAA GAT GTT GGT ATT AAT GGA AAA
-----
L  F  A  S  E  V  S  K  V  A  Q  D  V  G  I  N  G  K
C  L  H  Q  K  C  R  K  W  H  K  M  L  V  L  M  E  N
V  C  I  R  S  V  E  S  G  T  R  C  W  Y  *  W  K  I

1683      1692      1701      1710      1719      1728
TTA GGT ATT CAA GCA CAA GTT AGT GAT GTT GAT GGA TTA TCG AAG GAG ATT ACG
-----
L  G  I  Q  A  Q  V  S  D  V  D  G  L  W  K  E  I  T
*  V  F  K  H  K  L  V  M  L  M  D  Y  G  R  R  L  R
R  Y  S  S  T  S  *  *  C  *  W  I  M  E  G  D  Y  V

1737      1746      1755      1764      1773      1782
TCT AAT GTA AAT ACC ATG GCT TCA AAT TTA ACT TCG CAA GTG AGA GCT TTT GCA
-----
S  N  V  N  T  M  A  S  N  L  T  S  Q  V  R  A  F  A
L  M  *  I  P  W  L  Q  I  *  L  R  K  *  E  L  L  H
*  C  K  Y  H  G  F  K  F  N  F  A  S  E  S  F  C  T

1791      1800      1809      1818      1827      1836
CAG ATT ACT GCT GCT GCT ACT GAT GGG GAT TTC ACT AGA TTT ATT ACT GTT GAA
-----
Q  I  T  A  A  A  T  D  G  D  F  T  R  F  I  T  V  E
R  L  L  L  L  L  L  M  G  I  S  L  D  L  L  L  L  K
D  Y  C  C  C  Y  *  W  G  F  H  *  I  Y  Y  C  *  S

1845      1854      1863      1872      1881      1890
GCA CTG GGA GAG ATG GAT GCG TTG AAA ACA AAG ATT AAT CAA ATG GTG TTT AAC
-----
A  L  G  E  M  D  A  L  K  T  K  I  N  Q  M  V  F  N
H  W  E  R  W  M  R  *  K  Q  R  L  I  K  W  C  L  T
T  G  R  D  G  C  V  E  N  K  D  *  S  N  G  V  *  L

1899      1908      1917      1926      1935      1944
TTA AGG GAA TCG CTT CAA AGG AAT ACT GCG GCT AGA GAA GCT GCT GAG TTG GCC
-----
L  R  E  S  L  Q  R  N  T  A  A  R  E  A  A  E  L  A
*  G  N  R  F  K  G  I  L  R  L  E  K  L  L  S  W  P
K  G  I  A  S  K  E  Y  C  G  *  R  S  C  *  V  G  Q

```

1953 1962 1971 1980 1989 1998
 AAT AGT GCG AAA TCC GAG TTT TTA GCA AAC ATG TCG CAT GAG ATT AGA ACA CCA
 N S A K S E F L A N M S H E I R T P
 I V R N P S F * Q T C R M R L E H H
 * C E I R V F S K H V A * D * N T I
 2007 2016 2025 2034 2043 2052
 TTG AAT GGG ATT ATT GGT ATG ACA CAG TTG TCR CTT GAT ACA GAG TTG ACR CMG
 L N G I I G M T Q L S L D T E L T X
 E W D Y W Y D T V V X * Y R V D X V
 2061 2070 2079 2088 2097 2106
 TAC CAA CGA GAG ATG TTG TCG ATT GTG CAT AAC TTG GCA AAT TCC TTG TTG ACC
 Y Q R E M L S I V H N L A N S L L T
 T N E R C C R L C I T W Q I P C * P
 P T R D V V D C A * L G K F L V D H
 2115 2124 2133 2142 2151 2160
 ATT ATA GAC GAT ATA TTG GAT ATT TCT AAG ATT GAG GCG AAT AGA ATG ACG GTG
 I I D D I L D I S K I E A N R M T V
 L * T I Y W I F L R L R R I E * R W
 Y R R Y I G Y F * D * G E * N D G G
 2169 2178 2187 2196 2205 2214
 GAA CAG ATT GAT TTT TCA TTA AGA GGG ACA GTG TTT GGT GCA TTG AAA ACG TTA
 E Q I D F S L R G T V F G A L K T L
 N R L I F H * E G Q C L V H * K R *
 T D * F F I K R D S V W C I E N V S
 2223 2232 2241 2250 2259 2268
 GCC GTC AAA GCT ATT GAA AAA AAC CTA GAC TTG ACC TAT CAA TGT GAT TCA TCG
 A V K A I E K N L D L T Y Q C D S S
 P S K L L K K T * T * P I N V I H R
 R Q S Y * K K P R L D L S M * F I V
 2277 2286 2295 2304 2313 2322
 TTT CCA GAT AAT CTT ATT GGA GAT AGT TTT AGA TTA CGA CAA GTT ATT CTT AAC
 F P D N L I G D S F R L R Q V I L N
 F Q I I L L E I V L D Y D K L F L T
 S R * S Y W R * F * I T T S Y S * L
 2331 2340 2349 2358 2367 2376
 TTG GCT GGT AAT GCT ATT AAG TTT ACT AAA GAG GGG AAA GTT AGT GTT AGT GTG
 L A G N A I K F T K E G K V S V S V
 W L V M L L S L L K R G K L V L V *
 G W * C Y * V Y * R G E S * C * C E
 2385 2394 2403 2412 2421 2430
 AAA AAG TCT GAT AAA ATG GTG TTA GAT AGT AAG TTG TTG TTA GAG GTT TGT GTT
 K K S D K M V L D S K L L L E V C V
 K S L I K W C * I V S C C * R F V L
 K V * * N G V R * * V V V R G L C *

GGW = GGA/T (Gly)
 ACY = ACC/T (Thy)
 TCR = TCA/g (Ser)

490
 1980
 490
 1490
 3
 497A

2439 2448 2457 2466 2475 2484
AGC GAC ACG GGA ATA GGT ATA GAG AAA GAC AAA TTG GGA TTG ATT TTC GAT ACC

S D T G I G I E K D K L G L I F D T
A T R E * V * R K T N W D * F S I P
R H G N R Y R E R Q I G I D F R Y L

2493 2502 2511 2520 2529 2538
TTC TGT CAA GCT GAT GGT TCT ACT ACA AGA AAG TTT GGT GGT ACA GGT TTA GGG

F C Q A D G S T T R K F G G T G L G
S V K L M V L L Q E S L V V Q V * G
L S S * W F Y Y K K V W W Y R F R V

2547 2556 2565 2574 2583 2592
TTG TCA ATT TCC AAA CAG TTG ATA CAT TTA ATG GGT GGA GAG ATA TGG GTT ACT

L S I S K Q L I H L M G G E I W V T
C Q F P N S * Y I * W V E R Y G L L
V N F Q T V D T F N G W R D M G Y F

2601 2610 2619 2628 2637 2646
TCG GAG TAT GGA TCC GGR TCA AAC TTT TAT TTT ACG GTG TGC GTG TCG CCA TCT

S E Y G S G S N F Y F T V C V S P S
R S M D P X Q T F I L R C A C R H L
G V W I R X K L L F Y G V R V A I *

2655 2664 2673 2682 2691 2700
AAT ATT AGA TAT ACT CGA CAA ACC GAA CAA TTG TTA CCA TTT AGT TCC CAT TAT

N I R Y T R Q T E Q L L P F S S H Y
I L D I L D K P N N C Y H L V P I M
Y * I Y S T N R T I V T I * F P L C

2709 2718 2727 2736 2745 2754
GTG TTA TTT GTA TCG ACT GAG CAT ACT CAA GAA GAA CTT GAT GTG TTG AGA GAT

V L F V S T E H T Q E E L D V L R D
C Y L Y R L S I L K K N L M C * E M
V I C I D * A Y S R R T * C V E R W

2763 2772 2781 2790 2799 2808
GGA ATT ATA GAA CTT GGA TTG ATA CCT ATA ATA GTG AGA AAT ATT GAA GAT GCA

G I I E L G L I P I I V R N I E D A
E L * N L D * Y L * * * E I L K M Q
N Y R T W I D T Y N S E K Y * R C N

2817 2826 2835 2844 2853 2862
ACA TTG ACT GAG CCG GTG AAA TAT GAT ATA ATT ATG ATT GAT TCG ATA GAG ATT

T L T E P V K Y D I I M I D S I E I
H * L S R * N M I * L * L I R * R L
I D * A G E I * Y N Y D * F D R D C

2871 2880 2889 2898 2907 2916
GCC AAA AAG TTG AGG TTG TTA TCG GAG GTT AAA TAT ATT CCG TTG GTT TTG GTC

A K K L R L L S E V K Y I P L V L V
P K S * G C Y R R L N I F R W F W S
Q K V E V V I G G * I Y S V G F G P

2925 2934 2943 2952 2961 2970
CAT CAT TCT ATT CCA CAG TTG AAT ATG AGA GTA TGT ATT GAT TTG GGG ATA TCT

H H S I P Q L N M R V C I D L G I S
I I L F H S * I * E Y V L I W G Y L
S F Y S T V E Y E S M Y * F G D I F

2979 2988 2997 3006 3015 3024
TCC TAT GCA AAT ACG CCA TGT TCG ATC ACG GAC TTG GCC AGT GCG ATT ATA CCA

S Y A N T P C S I T D L A S A I I P
P M Q I R H V R S R T W P V R L Y Q
L C K Y A M F D H G L G Q C D Y T S

3033 3042 3051 3060 3069 3078
GCG TTG GAG TCG AGA TCT ATA TCA CAG AAC TCA GAC GAG TCG GTG AGG TAC AAA

A L E S R S I S Q N S D E S V R Y K
R W S R D L Y H R T Q T S R * G T K
V G V E I Y I T E L R R V G E V Q N

3087 3096 3105 3114 3123 3132
ATA TTA CTA GCA GAG GAC AAC CTC GTC AAT CAG AAA CTT GCA GTT AGG ATA TTA

I L L A E D N L V N Q K L A V R I L
Y Y * Q R T T S S I R N L Q L G Y *
I T S R G Q P R Q S E T C S * D I R

3141 3150 3159 3168 3177 3186
GAA AAG CAA GGG CAT CTG GTG GAA GTA GTT GAG AAC GGA CTC GAG GCG TAC GAA

E K Q G H L V E V V E N G L E A Y E
K S K G I W W K * L R T D S R R T K
K A R A S G G S S * E R T R G V R S

3195 3204 3213 3222 3231 3240
GCG ATT AAG AGG AAT AAA TAT GAT GTG GTG TTG ATG GAT GTG CAA ATG CCT GTA

A I K R N K Y D V V L M D V Q M P V
R L R G I N M M W C * W M C K C L *
D * E E * I * C G V D G C A N A C N

3249 3258 3267 3276 3285 3294
ATG GGT GGG TTT GAA GCT ACG GAG AAG ATT CGA CAA TGG GAG AAA AAG TCT AAC

M G G F E A T E K I R Q W E K K S N
W V G L K L R R R F D N G R K S L T
G W V * S Y G E D S T M G E K V * P

3303 3312 3321 3330 3339 3348
CCA ATT GAC TCG TTG ACG TTT AGG ACT CCA ATT ATT GCC CTC ACT GCA CAC GCC

P I D S L T F R T P I I A L T A H A
Q L T R * R L G L Q L L P S L H T P
N * L V D V * D S N Y C P H C T R H

3357 3366 3375 3384 3393 3402
ATG TTA GGT GAT AGA GAA AAG TCA TTG GCC AAG GGG ATG GAC GAT TAT GTG AGT

M L G D R E K S L A K G M D D Y V S
C * V I E K S H W P R G W T I M * V
V R * * R K V I G Q G D G R L C E *

3411 3420 3429 3438 3447 3456
AAG CCA TTG AAG CCG AAA TTG TTA ATG CAG ACG ATA AAC AAG TGT ATT CAT AAT
K P L K P K L L M Q T I N K C I H N
S H * S R N C * C R R * T S V F I I
A I E A E I V N A D D K Q V Y S * Y

3465 3474 3483 3492 3501 3510
ATT AAC CAG TTG AAA GAA TTG TCG AGA AAT AGT AGG GGT AGC GAT TTT GCA AAG
I N Q L K E L S R N S R G S D F A K
L T S * K N C R E I V G V A I L Q R
* P V E R I V E K * * G * R F C K E

3519 3528 3537 3546 3555 3564
AAG ATG ACC CGA AAC ACA CCC GGA AGC ACG ACC CGT CAG GGG AGT GAT GAG GGG
K M T R N T P G S T T R Q G S D E G
R * P E T H P E A R P V R G V M R G
D D P K H T R K H D P S G E * * G E

3573 3582 3591 3600 3609 3618
AGT GTA AAG GAC ATG ATT GGG GAC ACT CCC CGT CAA GGG AGT GTG GAG GGA GGG
S V K D M I G D T P R Q G S V E G G
V * R T * L G T L P V K G V W R E G
C K G H D W G H S P S R E C G G R G

3627 3636 3645 3654 3663 3672
GGT ACA AGT AGT AGA CCA GTA CAG AGA AGG TCT GCC AGG GAG GGG TCG ATC ACT
G T S S R P V Q R R S A R E G S I T
V Q V V D Q Y R E G L P G R G R S L
Y K * * T S T E K V C Q G G V D H Y

3681 3690 3699 3708 3717 3726
ACA ATT AGT GAA CAA ATC GAC CGT TAG CTA ACG ACT CAA GCT GAC GCT TGA GTC
T I S E Q I D R * L T T Q A D A * V
Q L V N K S T V S * R L K L T L E S
N * * T N R P L A N D S S * R L S Q

3735 3744 3753 3762 3771 3780
AAA GCT ACA AAT ATT TAG CCA ATT GTT TAC TTA GAT AAA TAA AAT ACA AGT AAA
K A T N I * P I V Y L D K * N T S K
K L Q I F S Q L F T * I N K I Q V N
S Y K Y L A N C L L R * I K Y K * T

3789 3798 3807 3816 3825 3834
CCA TTG TTG TGT TTA GAT CAA TAA TTG AAA AAT AAA CAA GAT TAC TAA AAA TAT
P L L C L D Q * L K N K Q D Y * K Y
H C C V * I N N * K I N K I T K N I
I V V F R S I I E K * T R L L K I S

3843 3852 3861 3870 3879 3888
CAA GCC AAA TTG TTG TTG CAG GAG CTG GGT TTT TGG GKG GGG TTA ACT TTT TTA
Q A K L L L Q E L G F W X G L T F L
K P N C C C R S W V F G X G * L F Y
S Q I V V A G A G F L G G V N F F T

540
486

134

1070AA

1250
1070
180x3
5403696
487

3009

136

1357

1062

3897 3906 3915 3924 3933 3942
CCA AAA AAT GGA TAA AAA AGG GGA TGT GAT CCA AGT AGT AAC TTT AGT GAC TGT
P K N G * K R G C D P S S N F S D C
Q K M D K K G D V I Q V V T L V T V
K K W I K K G M * S K * * L * * L F

3951 3960 3969 3978 3987 3996
TTA GGT TAC TTG AGC TAT CCA ATT AGA ATN TCM CCC CCC GCA GTA AGT TTG GTC
L G Y L S Y P I R X S P P A V S L V
* V T * A I Q L E X X P P Q * V W S
R L L E L S N * N X X P R S K F G L

4005 4014 4023 4032 4041 4050
TTA TTG TTT ACG GAA AAA TAA GAA ACC CTA GCC CTG GAC TAG CCC CTA CCT AGT
L L F T E K * E T L A L D * P L P S
Y C L R K N K K P * P W T S P Y L V
I V Y G K I R N P S P G L A P T * F

4059 4068 4077 4086 4095 4104
TTT GAA GTG AAA TTT TTT TTT TTT TAG TTG ACT CCC CCC CCC TAG ACC AAT
F E V K F F F F F * L T P P P * T N
L K * N F F F F F S * L P P P R P I
* S E I F F F F L V D S P P L D Q S

4113 4122 4131 4140 4149 4158
CGA AAG CCG TGG TAT TAT GCC GGG CAT AGA AAA AAA TCT TTC TTT TTT CTT TTT
R K P W Y Y A G H R K K S F F F L F
E S R G I M P G I E K N L S F F F F
K A V V L C R A * K K I F L F S F F

4167 4176 4185 4194 4203 4212
TTT GGT GTG GGC CCC CAG TTT ATG CAA CAT CAC TTA ACC CCC CTC AGC CAA AAA
F G V G P Q F M Q H H L T P L S Q K
L V W A P S L C N I T * P P S A K K
W C G P P V Y A T S L N P P Q P K K

4221 4230 4239 4248 4257 4266
AAA CCA TTT AAT TAT CCT TCC CCC CCC CCG CGG ATG AAG AAA ACC NAA CCA AAA
K P F N Y P S P P P R M K K T X P K
N H L I I L P P P P R G * R K P N Q K
T I * L S F P P P A D E E N X T K K

4275 4284 4293 4302 4311 4320
AAA ANT TIN TTT TTT TTT TCC CCN CCN CCT NTC TTT TCC AAC AAC CAC ATT TNC
K X X F F F S P P P X F S N N H I X
K X X F F F P X X L S F P T T T F X
X F X F F F P X X X L F Q Q P H X X

4329 4338 4347 4356 4365 4374
NTN TCG GAT CCC CCC ACC TGA TTT CAA ACT NAA AAN ACN GGC GGG CCT TTT TNT
X S D P P T * F Q T X X T G G P F X
X R I P P P D F K L K X X A G L F X
X G S P H L I S N X K X X R A F X F